

REMARKS

Entry of the foregoing, reexamination and further and favorable reconsideration of the above-identified application in view of the following remarks is respectfully requested.

The claims have been amended to be directed to an isolated "polynucleotide" rather than "gene." No new matter is added by this amendment. New claims 54-67 have been added. These claims are similarly directed to an isolated polynucleotide and vector, hosts, plants and cut flowers comprising same, as well as methods of using the claimed polynucleotide. No new matter is added by these claims. Support for the claims may be found at the very least at page 4, ln. 34 - page 5, ln. 7, page 5, lns. 17-29, page 6, lns. 32-34, and page 8, lns. 24-32.

Amendments to form of the claims, in particular the method claims, have also been made. These amendments do not alter the scope of the claims, but were made to put the claims in more proper format.

Claims 1-3, 5-12, 20, 22-45 and 48-53 have been rejected under 35 U.S.C. §112, first paragraph, as allegedly not being described in the specification. This rejection is respectfully traversed.

According to the Official Action, the description in the specification does not describe the genus of sequences being claimed. This rejection is believed to be in error. The instant application describes the cloning of many cDNA's which encode an enzyme having an aromatic acyl group transfer activity, as well as the cDNA's which have been cloned. For example, in Example 6 the applicants describe cDNA of gentian origin; in Example 8, cDNA of petunia origin is disclosed; and in Example 20, cDNA of lavender

origin is disclosed. The cDNA's disclosed in Examples 6, 8 and 20 were obtained using a hybridization method (described in the specification) to select desired cDNA. Example 11 describes a cDNA of Perilla origin and Example 12 describes a cDNA of cineraria origin. The cDNA's of both Examples 11 and 12 were obtained by using synthetic DNA primers.

At page 9, ln. 37 - page 10, ln. 29, the specification describes that by using the cDNAs specifically described in the specification, other cDNAs of acyltransferases can be obtained. Moreover, comparing the various cDNAs obtained, a region of conserved amino acid sequence was observed. *See*, SEQ ID NO. 21. Thus, the specification would describe to a person skilled in the art the genus of polynucleotides as claimed.

A specification may, within the meaning of 35 U.S.C. §112, first paragraph, contain a written description of a broadly claimed invention without describing all species. Utter v. Hiraga, 6 USPQ2d 1709, 1714 (Fed. Cir. 1988). Applicants have disclosed the amino acid sequences of numerous proteins, from various species, which they have isolated using their methods, and describe how one skilled in the art would obtain a protein having aromatic acyl transferase activity, as claimed. These descriptions are sufficient for purposes of the written description requirement of 35 U.S.C. § 112, first paragraph. Thus, the application provides written description support for the subject matter claimed.

It is believed at the very least that newly added claims 54-67 are allowable. These claims are directed to the isolated polynucleotides encoding an anthocyanin acyltransferase, which encodes an amino acid sequence selected from the amino acid sequences of SEQ ID Nos: 1 to 6. These claims are directed to the specific sequences disclosed in the specification.

In light of these remarks, applicants respectfully request withdrawal of this rejection under 35 U.S.C. § 112, first paragraph.

Claims 1-3, 5-12, 20, 22-45 and 48-53 have been rejected under 35 U.S.C. §112, first paragraph, as allegedly not being enabled by the specification. This rejection is respectfully traversed.

In the instant application, applicants teach how one skilled in the art could obtain proteins which have aromatic acyl group transfer activity. As discussed on pages 5 and 6 of the specification as filed, prior to the present invention all attempts to purify aromatic acyltransferases had failed. The inventors were the first to succeed in purifying this enzyme using various chromatographic techniques. The DNA obtained was sequenced, and then used as a probe. DNA encoding the amino acid sequences of SEQ ID Nos: 1-6 were then obtained. Using the known DNA as probes, and recognizing the conserved regions, additional DNA falling within the scope of the claims can be obtained, as described at the very least at pages 9-10. By using this method, one of skill in the art can thus isolate proteins which have aromatic acyl group transfer activity.

For example, using the amino acid sequence, one could develop a number of primers which could be used to amplify cDNA's from a cDNA library. Given that the genetic code is degenerative, one would have to develop a number of primers going in each direction (to cover all possible combinations of nucleic acids which would encode the amino acid segment). Using all possible combinations of the primers from each primer set (one set forward primer, one set reverse) one of skill in the art would then perform PCR on

cDNAs from a cDNA library. Any cDNA determined to have been amplified would then be further analyzed by sequencing to determine if the cDNA encodes the isolated protein.

For example, in Example 6 the applicants describe cDNA of gentian origin; in Example 8, cDNA of petunia origin is disclosed; and in Example 20, cDNA of lavender origin is disclosed. The cDNA's disclosed in Examples 6, 8 and 20 were obtained using a hybridization method as described in the specification to select desired cDNA. Example 11 describes a cDNA of perilla origin and Example 12 describes a cDNA of cineraria origin. The cDNA's of both Examples 11 and 12 were obtained by using synthetic DNA primers.

One of skill in the art could obtain a protein having an aromatic acyl group transfer activity of any origin using the methods described in Examples 6, 8, 11, 12 and 20. Example 3(6) teaches the probe which is used in Examples 6 and 8 to obtain a protein with aromatic acyl group transfer activity. Example 20 uses the same hybridization method as that taught in Example 3, but with a different flower species (i.e., *lavandula angustifolia* as opposed to *petunia hygrida* or *gentian*).

In Example 11, the applicants compared amino acid sequences from the proteins obtained in Examples 3, 6 and 8, and determined that a amino acid sequence was conserved between these proteins. They used this sequence to produce a primer which will amplify aromatic acyl transfer genes. The applicants next used this primer to amplify DNA from a cDNA library developed from perillas, and obtained a protein with aromatic acyl group transfer activity. In Example 12, the primer was also used to screen for genes in *Senecio cruentus*. Thus, the applicants have shown that this protein has a conserved region which

is found in all of the flower species discussed in the specification, and primers from this conserved region can be used to isolate proteins from other flower species.

These steps are all readily known and easily practiced by those skilled in the art. Enablement is not precluded by the necessity for some experimentation. However, experimentation needed to practice the invention must not be undue experimentation. The "key" word is undue, not experimentation. Although this may require a good deal of experimentation, the experimentation would not be undue to one of skill in the art. In fact, this type of experimentation would be commonplace for one of skill in the art. Therefore, it is believed that the claims are enabled by the specification, given what is known to one of skill in the art.

It is believed at the very least that newly added claims 54-67 are allowable. As stated *supra*, these claims are directed to the polynucleotides encoding the amino acid sequences of SEQ ID Nos: 1-6, as given in the specification. No undue experimentation would be required to practice the claimed inventions as recited therein.

In view of the above, withdrawal of the rejection of record is respectfully requested, and believed to be in order.

Claims 1-3, 5-8, 11, 20, 22-45 and 48-53 have been rejected under 35 U.S.C. §112, second paragraph, as allegedly not being described in the specification. This rejection is believed to be rendered moot by the instant amendments.

The claims have been amended, as helpfully suggested by the Examiner, to recite "polynucleotide" rather than "gene." Claims 20, 22, 23 and 33-35 were said to lack antecedent basis for the phrase "the protein produced." These claims have been amended

to provide antecedent basis. Claims 22, 23, 34 and 35 have also been rejected as reciting "incomplete method steps." The method claims have been rewritten to make it more clear that the steps are "complete." Claim 25 was said to be unclear. This claim has been amended as helpfully suggested by the Examiner. In claim 28, the claim has been amended to refer to the "amino acid sequence" of SEQ ID Nos: 1-6, and to amend "aryl" to "acyl." "Host" in claims 10-12 and 30-32 have been amended to "host cell," and "controlling" has been replaced with "altering", as suggested.

The rejection under §112(2) is thus respectfully believed to be moot in view of the instant amendments. Withdrawal of the rejection is respectfully requested and believed to be in order.


Applicants note with appreciation the indication that the claims are free of the prior art.

Further and favorable action in the form of a Notice of Allowance is respectfully requested. Such action is believed to be in order.

In the event that there are any questions relating to this application, it would be appreciated if the Examiner would telephone the undersigned at (508) 339-3684 concerning such questions so that prosecution of this application may be expedited.

Respectfully submitted,

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Marked-up Claims 1-3, 5-9, 20, 22, 23, 25, 28, 29, 33-36 and 46-53

1. (Five Times Amended) An isolated [gene] polynucleotide encoding an

anthocyanin acyltransferase.

2. (Amended) The [gene] polynucleotide according to claim 1 produced by the process of cloning using as a primer a nucleotide sequence encoding the amino acid sequence of SEQ ID No. 21.

3. (Amended) The [gene] polynucleotide according to claim 2 wherein said primer has the nucleotide sequence of SEQ ID No. 22.

5. (Three Times Amended) The [gene] polynucleotide according to claim 1 encoding a protein, which [gene] polynucleotide hybridizes with [a consensus region] a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 21 or all of the nucleotide sequence encoding any of the amino acid sequences of SEQ ID No. 1 to 6 under the condition of 5 x SSC and 50°C, and which protein transfers an aromatic acyl group to flavonoid.

6. (Three Times Amended) The [gene] polynucleotide according to claim 1 encoding a protein, which [gene] polynucleotide hybridizes with [a consensus region] a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 21 or all of the



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Marked-up Claims 1-3, 5-9, 20, 22, 23, 25, 28, 29, 33-36 and 46-53

nucleotide sequence encoding any of the amino acid sequences of SEQ ID No. 1 to 6 under the condition of 2 x SSC and 50°C and which protein transfers an aromatic acyl group to flavonoid.

7. (Four Times Amended) The [gene] polynucleotide according to claim 1 encoding a protein which consists of an amino acid sequence which is at least 30% homologous to any one of the amino acid sequences of SEQ ID No. 1 to 6, and which transfers an aromatic acyl group to flavonoid.

8. (Three Times Amended) The [gene] polynucleotide according to claim 1 encoding a protein which has an amino acid sequence having a homology of at least 69% with any of the amino acid sequences of SEQ ID No. 1 to 6, and which transfers an aromatic acyl group to flavonoid.

9. (Amended) A vector comprising a [gene] polynucleotide according to claim 1.

10. (Amended) A host cell transformed with a vector according to claim 9.

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Marked-up Claims 1-3, 5-9, 20, 22, 23, 25, 28, 29, 33-36 and 46-53

11. (Amended) A host cell according to claim 10 wherein said host is a microbial or animal cell.
12. (Amended) A host cell according to claim 10 wherein said host is a plant cell or a plant body.
20. (Amended) A method for acylating a pigment in a plant, comprising [the steps of] introducing a [gene] polynucleotide according to claim 1 into the plant, [allowing] whereby said [gene] polynucleotide [to express] expresses a protein, and said protein acylates [acylate] the pigment in the plant [with the protein produced].
22. (Amended) A method for stabilizing a pigment in a plant, comprising [the steps of] introducing the [gene] polynucleotide according to claim 1 into a plant, whereby [allowing] said [gene] polynucleotide [to express] expresses a protein, and said protein acylates [acylate] the pigment in the plant [with the protein produced], which acylation stabilizes said pigment in the plant.
23. (Amended) A method for [controlling] altering the color of flowers, comprising [the steps of] introducing the [gene] polynucleotide according to claim 1 into a plant, [allowing] whereby said [gene] polynucleotide [to express] expresses a protein, and

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Marked-up Claims 1-3, 5-9, 20, 22, 23, 25, 28, 29, 33-36 and 46-53

said protein acylates [acylate] the pigment in the plant [with the protein produced], which acylation alters the color of flowers of said plant.

25. (Amended) A plant, a progeny or tissues thereof, each of whose color has been [controlled] altered by introducing therein to a [gene] polynucleotide according to claim 1[, or its progeny which has its color controlled, or tissues thereof].

28. (Four Times Amended) An isolated [gene] polynucleotide encoding an anthocyanin acyltransferase, which [gene] polynucleotide encodes an amino acid sequence selected from the group consisting of the amino acid sequences as set forth in SEQ ID No. 1 to 6, or hybridizes with a nucleotide sequence complementary to a nucleotide sequence selected from the group consisting of the nucleotide sequences encoding the amino acid sequences as set forth in SEQ ID No. 1 to 6 under the condition of 5 x SSC and 50°C or the condition of 2 x SSC and 50°C, and which [protein] anthocyanin acyltransferase transfers an aromatic [aryl] acyl group to flavonoid.

29. (Amended) A vector comprising a [gene] polynucleotide according to claim 28.

30. (Amended) A host cell transformed with a vector according to claim 29.

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Marked-up Claims 1-3, 5-9, 20, 22, 23, 25, 28, 29, 33-36 and 46-53

31. (Amended) A host cell according to claim 30 wherein said host is a microbial or animal cell.
32. (Amended) A host cell according to claim 30 wherein said host is a plant cell or a plant body.
33. (Amended) A method for acylating a pigment in a plant, comprising [the steps of] introducing a [gene] polynucleotide according to claim 28 into the plant, [allowing] whereby said [gene] polynucleotide [to express] expresses a protein, and said protein acylates [acylate] the pigment in the plant [with the protein produced].
34. (Amended) A method for stabilizing a pigment in a plant, comprising [the steps of] introducing the [gene] polynucleotide according to claim 28 into a plant, whereby [allowing] said [gene] polynucleotide [to express] expresses a protein, and said protein acylates [acylate] the pigment in the plant [with the protein produced], which acylation stabilizes said pigment in the plant.
35. (Amended) A method for [controlling] altering the color of flowers, comprising [the steps of] introducing the [gene] polynucleotide according to claim 28 into a plant, whereby [allowing] said [gene] polynucleotide [to express] expresses a protein, and

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Marked-up Claims 1-3, 5-9, 20, 22, 23, 25, 28, 29, 33-36 and 46-53

[acylating] said protein acylates the pigment in the plant [with the protein produced], which alters the color of flowers of said plant.

36. (Amended) A plant, a progeny or tissues thereof, each of whose color has been [controlled] altered by introducing thereinto a [gene] polynucleotide according to claim 28[, or its progeny having the same property, or tissues thereof].

46. (Twice Amended) The [gene] polynucleotide according to claim 1, wherein the anthocyanin acyltransferase transfers an aromatic acyl group to the glucose of the 3 or 5 position of anthocyanin.

47. (Twice Amended) The [gene] polynucleotide according to claim 2, wherein the [gene] polynucleotide encodes an anthocyanin acyltransferase which transfers an aromatic acyl group to the glucose at the 3 or 5 position of anthocyanin.

48. (Twice Amended) The [gene] polynucleotide according to claim 5, wherein the anthocyanin acyltransferase transfers an aromatic acyl group to the glucose at the 3 or 5 position of anthocyanin.

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Marked-up Claims 1-3, 5-9, 20, 22, 23, 25, 28, 29, 33-36 and 46-53

49. (Twice Amended) The [gene] polynucleotide according to claim 7, wherein the anthocyanin acyltransferase transfers an aromatic acyl group to the glucose at the 3 or 5 position of anthocyanin.

50. (Twice Amended) The [gene] polynucleotide according to claim 8, wherein the anthocyanin acyltransferase transfers an aromatic acyl group to the glucose at the 3 or 5 position of anthocyanin.

51. (Twice Amended) The [gene] polynucleotide according to claim 28, wherein the anthocyanin acyltransferase transfers an aromatic acyl group to the glucose at the 3 or 5 position of anthocyanin.

52. (Twice Amended) The [gene] polynucleotide according to claim 42, wherein the [gene] polynucleotide encodes an anthocyanin acyltransferase which transfers an aromatic acyl group to the glucose at the 3 or 5 position of anthocyanin.

53. (Twice Amended) An isolated acyltransferase [gene] polynucleotide which encodes an anthocyanin acyltransferase.